

SCREENING AND OPTIMISING METAL SALT CONCENTRATION FOR
MARINE MICROALGAE HARVESTING BY FLOCCULATION

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ABSTRACT

Biodiesel is one of the most renewable fuels that are non-toxic and biodegradable. Demand of biodiesel is constantly increasing as the reservoir of fossil fuel are depleting. The microalgae biomass with high oil content is significant as a sustainable resource for biodiesel production. Production of biodiesel using microalgae biomass appears to be a feasible alternative because there is no conflict with food supply compared with the first generation biofuels, such as oil crops and animal fat. This report deals with the screening and optimisation of metal salts for harvesting marine microalgae by flocculation. The metal salts studies are ferric chloride, aluminium sulphate and ferric sulphate. Wild *Nannochloropsis* strains of microalgae were cultivated aseptically in sea water for 7 days, after that the microalgae was harvested by using flocculation step with different concentration of metal salt. In order to monitor the efficiency of the metal salt, the turbidity region of microalgae in glass cylinder before and after flocculation was observed. Besides that cell dry weight and FAME (Fatty Acid Methyl Ester) produced was also compared for three flocculation agent used. The most efficient metal salt was then further optimized for its best performed concentration and pH. Chloride salts (FeCl_3) was found to be more efficient in comparison with sulfate salts ($\text{Al}_2(\text{SO}_4)_3$ and $\text{Fe}_2(\text{SO}_4)_3$) in harvesting microalgae. FeCl_3 gives the highest flocculation efficiency, cell dry weight and FAME production which are 99.3 %, 0.0791g, 44.3 % at 1.0 M concentration of FeCl_3 respectively. Ferric Chloride was further optimized, where the optimum pH and concentration of FeCl_3 are 7.5 and 0.9 M, with flocculation efficiency of 89.3 %, cell dry weight of 3.5 g and FAME production of 43.3 %. In conclusion 0.9 M ferric chloride salt at pH 7.5 is optimum in harvesting microalgae by flocculation.

ABSTRAK

Biodiesel adalah salah satu bahan api yang boleh diperbaharui yang bukan toksik dan terbiodegradasi. Permintaan biodiesel sentiasa meningkat kerana bahan api fosil yang semakin berkurangan. Biojisim mikroalga yang mengandungi kandungan minyak yang tinggi adalah penting sebagai sumber bagi pengeluaran biodiesel. Pengeluaran biodiesel menggunakan bio jisim mikroalga muncul sebagai sumber alternatif yang boleh dilaksanakan kerana tiada konflik dengan bekalan makanan berbanding dengan biofuel generasi pertama, seperti minyak dari tanaman dan lemak haiwan. Laporan ini membincangkan penapisan dan pengoptimuman untuk penuaian mikroalga laut oleh pemberbukuan. Garam logam yang dikaji adalah ferric klorida, aluminium sulfat dan ferric sulfat. Jenis *Nannochloropsis* liar mikroalga dibiakkan di dalam air laut selama 7 hari, selepas itu mikroalga akan dituai dengan menggunakan langkah pemberbukuan dengan perbezaan kepekatan garam logam. Dalam usaha untuk memantau kecekapan garam logam, kawasan kekeruhan mikroalga dalam silinder kaca sebelum dan selepas pemberbukuan diperhatikan. Selain daripada itu, berat sel kering dan FAME (Fatty Acid Methyl Ester) produktiviti yang dihasilkan juga telah dibandingkan untuk tiga ejen yang digunakan. Garam logam yang paling kecekapan akan diteruskan pengkajiannya bagi mengoptimumkan hasil yang paling baik. Garam klorida (FeCl_3) adalah lebih cekap berbanding dengan garam sulfat ($\text{Al}_2(\text{SO}_4)_3$ dan $\text{Fe}_2(\text{SO}_4)_3$) untuk penuaian mikroalga. FeCl_3 memberikan pemberbukuan kecekapan tertinggi, berat sel kering dan FAME produktiviti adalah seperti 99.3%, 0.0791g, 44.3% pada kepekatan 1.0 M FeCl_3 . FeCl_3 telah dipilih untuk diulangkaji semula bagi mengoptimumkannya, dimana pH optimum dan kepekatan FeCl_3 pada 7.5 pH dan 0.9M dengan kecekapan pemberbukuan 89.3%, sel berat kering 3.52905g dan FAME produktiviti 43.29%. Kesimpulannya, 0.9 M ferric klorida garam pada pH 7.5 adalah yang paling optimum untuk penuaian mikroalga oleh pemberbukuan.

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LIST OF ABBREVIATIONS

Fe	Iron
FeCl ₃	Ferric Chloride
GC	Gas Chromatography
GHG	Greenhouse Gases
CDW	Cell Dry Weight
FE	Flocculation Efficiency
NaOH	Sodium Hydroxide / Natrium Hydroxide
pH	Hydrogen Ion Concentration
r	Error
rpm	Revolutions per minute
V	Volume
Al ₂ (SO ₄) ₃	Aluminium Sulphate
Fe ³⁺	Ion Iron (III) / Ion Ferric (III)
FAME	Fatty Acid Methyl Ester
Ave	Average

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Petroleum is unsustainable source fuel because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Biofuels production is expected to propose new opportunities to diversify income and fuel supply sources, to encourage employment in rural areas, to build up long term substitution of fossil fuels, and to reduce greenhouse gas (GHG) emissions, boosting the decarbonisation of transportation fuels and raising the security of energy supply. Additionally, biodiesel claims to have lower environmental impacts and ensure the same level of performance of existing fuels. So it can be said that biodiesel deserves as one of the most renewable fuels because of having superiority as non-toxic and biodegradable (Mata *et al.*, 2010).

The potential of microalgae as a source of biofuel is subject to intense academic and industrial research. Microalgae feedstock are gaining interest in today's energy scenario due to their fast growth potential along with relatively high lipid, carbohydrate and nutrients contents (Singh and Gu, 2010).

Microalgae use sunlight to produce oils by convert carbon dioxide. It is more efficient than crop plants because the production of oil from many microalgae greatly exceeds the oil production of the best producing oil crops. Microalgae can provide several different types of potential renewable biofuels such as methane produced by anaerobic digestion of the algal biomass. In addition microalgae have potential in production of foods, feeds, and high-value bioreactives (Chisti, 2007).

1.2 Problem Statement

Biodiesel production is expected to offer the lower or same price as existing fuels. But to achieve the target, the microalgae biodiesel value chain needs to consider such as: microalgae strain, microalgae cultivation unit, microalgae cultivation, site selection, microalgae harvesting and biomass concentration, microalgae processing and components extraction, biodiesel production. Any improvement of these value chain components will give some profit to lower the cost-price of microalgae biodiesel.

The harvest of the microalgae biomass is a very significant process after microalgae cultivation. Various flocculation methods could be applied in microalgae biomass recovery. However, economic cost and environmental problem should be considered especially in view of the production of microalgae biomass in large scale. Some flocculants such as multivalent metal salts would cause water pollution. Therefore, the objective of flocculation efficiency will have to be achieved by using the least amount of flocculant possible.

In this study, microalgae harvest will be focused. Harvesting of microalgae biomass involves one or more solid–liquid separation steps. Existing technologies such as centrifugation, filtration, flocculation, dissolved air floatation, and sedimentation are well suited to harvesting small particles from bulk. However, these processes are either too energy or chemical-intensive or require too much time, to be practical for harvesting lipid-rich microalgae.

1.3 Research Objective

The main objectives of this research are as follows:

- To screen an efficient metal salts for harvesting microalgae by flocculation
- To optimise the concentration metal salt and pH in flocculation step.

1.4 Scope of Study

Flocculation is one of the effective and inexpensive methods for harvesting microalgae in large scale. In this study, ferric chloride, aluminium sulphate and ferric sulphate were screened for their ability to efficiently flocculate the microalgae. This will be done by comparing the efficiency of flocculation and its cell dry weight. The amount of oil in the microalgae biomass would be reacted to form FAME and analyzed by Gas Chromatograph- Flame Ionization Detector (GC-FID). Best performed metal salts were further optimized in order to determine the optimum concentration and pH to efficiently flocculate the microalgae.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microalgae

There are several different types of microalgae exists in this world such as blue green algae (*Cyanobacterium*), *Spirulina* (*Arthrospira*) *Platensis geitler*, *Synechococcus synechocystis*, yellow green algae (*Xanthophytes*) *Olisthodiscus*, red algae (*Rhodophytes*) *Corallina*, golden algae (*Chrysophyte*), brown algae (*Eustigmatophyceae*) *Nannochloropsis gaditana*, green algae, *Haematococcus pluvialis*, *Chlamydomonas*, *Dunaliella* (Raja et al., 2008). The term microalgae refers to the aquatic microscopic plants (organisms with chlorophyll α and a thallus not differentiated into roots, stem and leaves), and the oxygenic photosynthetic bacteria. While by referring algae as feedstocks for biofuels, the definition includes all unicellular and simple multi-cellular microorganisms, including both prokaryotic microalgae, e.g. *cyanobacteria* (*Chloroxybacteria*), and eukaryotic microalgae, e.g. green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*) (Singh and Gu, 2010). Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow fast and live in severe conditions due to their unicellular or simple multicellular structure (Mata et al., 2010).

Microalgae can be utilized to produce various natural products. Microalgae are the profit resource with more than 25,000 species (Raja et al., 2008). Microalgae currently exist in earth ecosystems, not just in water but also land, representing a big diversity of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed (Mata et al., 2010).

During the past decades remarkable collections of microalgae have been observed by researchers in different countries. This collection declare to the large variety of different microalgae available to be selected for use in a broad diversity of applications, such as value added products for pharmaceutical purposes, food crops for human consumption and as energy source (Mata et al., 2010).

2.2 Biodiesel

Biodiesel is a combination of fatty acid alkyl esters obtained by transesterification (ester exchange reaction) of vegetable oils or animal fats. These lipid feedstocks are composed by 90–98% (weight) of triglycerides and small amounts of mono and diglycerides, free fatty acids (1–5%), and remaining amounts of phospholipids, phosphatides, carotenes, tocopherols, sulphur compounds, and traces of water (Mata et al., 2010) .

Biodiesel as a fuel gives much lower toxic air emissions than fossil diesel. Its chemical structure is Fatty Acid Alkyl Esters (FAAE). In addition, it gives cleaner burning and has less sulfur content, and thus reducing emissions. Almost all biodiesel is produced using base catalyzed transesterification as it is the most economical process requiring only low temperatures and pressures and producing a 98% conversion yield (Maan Hayyan et al., 2010).

Today a constant increasing demand of biodiesel can be observed against a clear depletion of fossil fuel reservoir. In other word, biodiesel demand is constantly increasing as the reservoir of fossil fuel are depleting. To solve this problem, lots of research had been done to replace the fossil fuel and as the alternative, microalgae were selected to overcome the biodiesel demand. Microalgae have high productivity and higher oil content compared to crops (up to 80% on dry weight); it is obvious the potential contribution that microalgae can give on large scale fuel oils production. A further profit is that microalgae may also be grown on dry lands unsuitable for conventional agriculture, such as desert areas, or in large reservoirs of saline water (Converti et al., 2009).

One of the major benefits of biodiesel compared to other alternative transportation fuels is that it can be used in existing diesel engines without modification, and can be blended in at any ratio with petroleum diesel. Usage of biodiesel will allow a balance to be required between agriculture, economic development and the environment. (Khan et al., 2009)

Table 2.1 shows the physical and chemical properties of a typical biodiesel. From table 2.1, it can be seen that biodiesel is more biodegradable which essentially frees of sulphur and aromatics, producing lower exhaust emissions than conventional diesel fuels (Gerpen, 2005). In other word, biodiesel is environmentally friendly energy sources than conventional diesel. Besides that, it can be seen biodiesel, have similar characteristics of petro-diesel oil which allows it's without any engine modification and it is suitable for blending in any ratio with petroleum diesel (Singh and Gu, 2010).

Table 2.1: Physical and Chemical Properties of Biodiesel (Demirbas, 2008)

Name	Biodiesel
Chemical Name	Fatty acid Methyl Ester
Chemical Formula Range	C14-C24 methyl esters
Kinematic Viscosity Range	3.3- 5.2
Density Range	860-894
Boiling point Range (K)	>475
Flash Point Range (K)	430-455
Distillation Range (K)	470-600
Vapor Pressure (mmHg at 295K)	<5
Solubility in water	Insoluble in water
Physical appearance	Light to dark yellow transparent liquid
Odor	Light soapy and oily odor
Biodegradability	More than conventional diesel
Reactivity	Stable, avoid strong oxidize agents

2.3 Microalgae as Potential Source of Biodiesel

Presently biodiesel is produced from plant and animal oils, but not from microalgae. This is likely to change as quite a lot of companies are attempting to commercialize microalgae biodiesel (Chisti, 2007). Nowadays, microalgae are seen as an option of feedstock for biodiesel production. It is the target of a large number of consortiums, private and public organizations' investments in R&D, who aiming to use the most valuable and cheap technology to produce large amounts of oil. Microalgae can offer feedstock for several diverse types of renewable fuels such as biodiesel, methane, hydrogen and ethanol (Mata et al., 2010).

Microalgae can afford several different types of renewable biofuels. These include methane formed by anaerobic digestion of the algal biomass; biodiesel derived from microalgae oil and photobiologically produced biohydrogen. The idea of using microalgae as a source of fuel is not new, but it is now being taken seriously because of increasing price of petroleum and, more significantly, the emerging concern about global warming that is related with burning of fossil fuels (Khan et al., 2009). The microalgae for biodiesel production can potentially use part of carbon dioxide from industrial plants; from this point of observation the microalgae can also be seen as simple CO₂ sequestrants to use in plants for green house gas emissions control (Converti et al., 2009).

Table 2.2 shows the oil contents in different type of microalgae, with a clear potential of microalgae to produce oil (Chisti, 2007). From table 2.2, it can be seen that *Schizochytrium* sp. gives the highest oil content. The species used in this study, however can produce quite comparable oil content too.

Table 2.2: Oil Content of Microalgae (Chisti, 2007)

Microalga	Oil content (% dry wet)
Botryococcus braunii	25–75
Chlorella sp	28–32
Cryptocodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp	25–33
Monallanthus salina	>20
Nannochloris sp	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp	50–77
Tetraselmis suecica	15–23
B. braunii	25–75

2.4 Advantage of the Microalgae Biodiesel

Many research reports and articles described many advantages of using microalgae for biodiesel production in comparison with other available feedstocks. The advantages of microalgae over higher plants as a source of transportation biofuels are numerous:

- Microalgae produce and gather large quantities of neutral lipids/oil [20–50% cell dry weight (CDW)] and grow at high rates (e.g. 1–3 doublings/day).
- Oil yield per area of microalgae cultures could significantly beat the yield of best oil seed crops.
- Microalgae can be cultivated in saline/brackish water/coastal seawater on non-arable land, and do not compete for resources with conventional agriculture.

- d) Microalgae tolerate marginal lands (e.g. desert, arid and semiarid lands) that are not suitable for regular agriculture.
- e) Microalgae utilize nitrogen and phosphorus from a diversity of wastewater sources (e.g. agricultural run-off, concentrated animal feed operations, and industrial and municipal wastewaters), providing the further benefit of wastewater bioremediation.
- f) Microalgae sequester CO₂ from flue gases emitted from fossil fuel-fired power plants and other sources, thus reducing emissions of a major green house gas. 1 kg of algal biomass requiring about 1.8 kg of CO₂.
- g) Microalgae produce value-added co-products or by-products (e.g. biopolymers, proteins, polysaccharides, pigments, animal feed and fertilizer) and does not need herbicide and pesticide.
- h) Microalgae grow in suitable culture vessels (photobioreactors) throughout the year with higher annual biomass productivity on an area basis (Khan et al., 2009).

From a practical point of analysis, microalgae are easy to cultivate, can grow with little or even no attention, using water unsuitable for human consumption and easy to obtain nutrients.

2.5 Microalgae Harvest

The production of biodiesel from microalgae involves microalgae cultivation, harvesting and processing. Algae harvesting consists of biomass recovery from the culture medium and its cost may contribute between 20 to 30% of the total biomass production cost (Molina et al., 2003). Therefore, it is important to choose algae with properties that simplify harvesting, like algae with large cell size or high specific gravity. This is an expensive part of industrial production of biomass. Microalgae have a very nice green-looking suspension. The optimal cell dry weight for industrial conversion is obtaining at least 300-400 g cell dry weight/ L of culture volume.

The cultivation of algae is nevertheless a complex process. The nutrient level in the water is required to be in a specific range and the pH must always be under control.

Nutrients must be controlled so algae could not be “starved”, and so that nutrients cannot be wasted either. Light needed to be neither too strong nor too weak. Algae only required 10% the amount of light they receive from direct sunlight (Barbosa, 2003).

There is no sole best method for harvesting microalgae. The choice of preferable harvesting technology depends on algae species, growth medium, algae production, end product, and production cost-benefit (Shelef et al., 1984). In order to eradicate large quantities of water and process large algae biomass volumes, an appropriate harvesting method may involve one or more steps and may be achieved in several physical, chemical, or biological ways, in order to perform the desired solid-liquid separation. Most common harvesting methods include sedimentation, centrifugation, filtration, and ultra-filtration, sometimes with an additional flocculation step or with a grouping of flocculation-flotation (Mata et al., 2010). Any suitable harvesting method must be able to process the large volume typical of the algae biomass production processes (Molina et al., 2003).

2.5.1 Centrifugation

Centrifugation is a method of separation algae by using a centrifuge to settling it into the bottom of the tank. This method may verify useful on a commercial and industrial scale, but is costly for personal use. A centrifuge is a device that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. The centrifuge works by the sedimentation principle.

Centrifugation has been applied successfully for preparing concentrates, but it does have some limitations. First, the procedure involves exposing cells to high gravitational and shear forces which damage the cell structure. Second, the processing of large culture volume can be time-consuming and require costly equipment, i.e. a specialised continuous centrifuge (Knuckey et al., 2006).

A further consideration in selecting a suitable harvest method is the acceptable level of moisture in the product. Gravity sediment sludge is generally more dilute than centrifugally recovered biomass. Too much moisture in the harvested biomass can

substantially manipulate the economics of product recovery in further downstream process, if dehydration of the biomass is required after harvesting (Molina et al., 2003).

2.5.2 Filtration

This method is carried out commonly on membranes of modified cellulose with the aid of a suction pump. The greatest advantage of this method is that it is able to collect microalgae cells with very low density. However, concentration by filtration is restricted to small volumes with eventual clogging in the filters by the package cells when vacuum is applied. Filter presses operating under pressure or vacuum are satisfactory for this purpose. It is relatively best suited for large algae such as *Coelastrum proboscideum* and *Spirulina platensis* but cannot recover organisms approaching bacterial dimensions (e.g., *Scenedesmus*, *Dunaliella*, *Chlorella*) (Shelef and Soeder, 1980). Membrane microfiltration and ultra-filtration are possible alternatives to conventional filtration for recovering algal biomass, which are more suitable for fragile cells and small-scale production processes. However, these filtration processes are more costly especially because of the need for membrane replacement and pumping (Mata et al., 2010).

2.5.3 Flocculation

Flocculation is the coalescence of finely divided suspended solids into larger loosely packed conglomerates, a process used widely in industry to remove suspended solids. In general, the first stage of flocculation is the aggregation of suspended solids into larger particles resulting from the interaction of the flocculant with the surface charge of the suspended solids. The second stage involves the coalescing of aggregates into large flocs that settle out of suspension (Knuckey et al., 2006).

The flocculation mechanism depends on cell and flocculant charges. Numerous chemical coagulants or flocculants have been tested in the literature (Papazi et al. 2010). The effluent algal suspension needs to be concentrated. It is proceed with flocculation and flotation in combination. However, the cell wall is quite a considerable barrier to facilitate the extraction and the thickness of the cell wall is affected by the conditions of

the cells at the time of harvesting. The disadvantage of several published processes for flocculating algae is that the harvested cells are difficult to disaggregate back to single cells, which is a requirement for feeding them to filter-feeding species such as bivalves (Knuckey et al., 2006).

Microalgae cells hold a negative charge that prevents aggregation of cells in suspension. The surface charge can be neutralized or reduced by adding flocculants such as multivalent cations and cationic polymers to the broth. Preferably, the flocculants used should be inexpensive, non-toxic, and effective in low concentrations. In addition, the flocculants should be selected so that further downstream processing is not adversely affected by its use (Mata et al., 2010).

Various methods of flocculation can be used to aggregate the microalgae cells to increase the effective 'particle' size and hence ease sedimentation, centrifugal recovery, and filtration (Elmaleh et al., 1991). For the large scale, flocculation is preferred for harvesting due to its low costs compared to other methods (Bilanovic et al., 1988). The flocculation process has been applied in the microalgae biomass recovery (Molina et al., 2003; Knuckey et al., 2006).

The properties of cellular surface, pH of the growth medium, concentration of the coagulant–flocculant, ionic strength of the culture solution, and the number of cells per unit volume are the major factors that influence coagulating–flocculating reactions in microalgae cultures and thereby harvesting of algal biomass (Bilanovic et al. 1998; Papazi et al., 2010). Harvesting of algal cells by coagulation involves pH adjustment or electrolyte addition, whereas flocculation involves addition of cationic polymers. Such approaches are quite convenient, because they allow rapid treatment of large quantities of microalgae cultures (Oh *et al.* 2001; Papazi et al., 2010).

Multivalent metal salts are effective flocculants or coagulants. Metal salts (aluminium sulphate, ferric chloride, ferric sulphate) are generally preferred in flocculation processes, because they lead to improved harvesting efficiency (Molina *et al.*, 2003; Papazi et al., 2010).

Among the three common metal salt used, the most efficiency is ferric chloride. After adding ferric chloride, the colour of microalgae will change from green to brown. For effective flocculation with FeCl_3 , the solution pH must be above 4 because no flocculation observed when $\text{pH} < 4$. Inefficient flocculation observed when insufficient FeCl_3 is added. Flocculation efficiency improves with increasing FeCl_3 concentration (Wyatt et al., 2011). Mineral coagulants such as alum and ferric chloride might be toxic to animals when consumed due to high concentration of residual aluminium and iron in the biomass harvested (Buelna et al., 1990).

The efficiency of electrolytes to induce coagulation is measured by the critical coagulation concentration, or the concentration required causing rapid coagulation. Coagulation efficiency of metal ions increases with increasing ionic charge (Molina et al., 2003). Poly-ferric sulphate (PFS) is observed to be a better flocculant compared to the more traditional non-polymerised metal salt flocculants (Jiang et al., 1993). This method is often too costly in large dimensions. However, interrupting the carbon dioxide supply to an algae system can cause microalgae to flocculate on its own, which is called “auto-flocculation”. Nutrient and CO_2 limitation and high pH and photosynthetic activity are believed to be important parameters in auto flocculation (Becker 1994).

Pre-polymerised metal salts are effective over a wider pH range than non-polymerised salts. An alternative to using metal salts is the use of cationic polymers (poly-electrolytes) (Tenney et al., 1969). Cationic polymers doses of between 1 and 10 mg ml^{-1} can induce flocculation of freshwater algae; however, a high salinity of the marine environment can restrain flocculation by poly-electrolytes (Bilanovic et al., 1988; Molina et al., 2003). In addition to reducing or neutralizing the surface charge on cells, the polymer flocculants can bring particles together by physically linking one or more particles through a process called bridging. Tenney et al. (1969) and Tilton et al. (1972) have demonstrated that the bridging mechanism also applies to flocculation of algal cells.

The flocculation effectiveness of poly-electrolytes relies on many factors, such as the molecular mass of the polymer, the charge density on the molecule, the dose

used, the concentration of the biomass, the ionic strength and pH of the broth, and the extent of mixing in the fluid. Generally, high molecular weight poly-electrolytes are better bridging agents. Similarly, a high charge density tends to unfold the polymer molecule, improving its bridging implementation and the ability to neutralize the surface charge on cells. A high cell concentration in the broth helps flocculation, because the cell–cell encounters are more frequent in concentrated suspensions (Flickinger and Drew, 1999; Molina et al., 2003).

2.6 Algae Oil Extraction

Under optimal conditions of growth, algae synthesize fatty acids for esterification into glycerol-based membrane lipids, which constitute about 5-20% of their dry cell weight. Fatty acids can be classified in medium chain (C10-C14), long chain (C16-C18) and very long chain (>C20) species and fatty acids derivatives. However, under unfavourable environmental conditions, many algae alter their lipid biosynthetic pathways to the formation and accumulation of neutral lipids (20-50% CDW), mainly in the form of triglycerides (TAGs) (Hussain et. al. 2010). For biodiesel production, these neutral lipids have to be extracted from microalgae biomass. Extraction algal oil is one of the most costly processes which can determine the sustainability of microalgae-based biodiesel. It is common to apply dehydration of algal biomass to increase its shelf-life and for the final product. Several methods have been employed to dry microalgae, where the most common include spray-drying, drum-drying, freeze-drying and sun-drying. After drying it follows the cell disruption of microalgae. Several methods can be used depending on the microalgae wall and on the product to be obtained. For biodiesel production, lipids and fatty acids have to be extracted from the microalgae biomass. Algal oil can be extracted using chemical methods or mechanical methods:

2.6.1 Mechanical Methods

These methods are classified a mechanical expeller press and ultrasonic assisted extraction.

2.6.1.1 Expeller Press

Algae are dried to retain its oil content and it can be pressed out with an oil press. Commercial manufactures use a combination of mechanical press and chemical solvents in extracting oil.

2.6.1.2 Ultrasonic Extraction

Ultrasonic waves are used to create bubbles in a solvent material, when these bubbles collapse near the cell walls, it creates shock waves and liquid jets that cause those cells walls to break and release their contents into the solvent. This method can be done with dry or wet microalgae, when using wet microalgae, it is necessary to extract part of the water from the smash before extraction oils with a solvent.

2.6.2 Chemical Methods

Neutral lipids or storage lipids are extracted with non-polar solvents such as diethyl ether or chloroform but membranes associated lipids are more polar and require polar solvents such as ethanol or methanol to disrupt hydrogen bonding or electrostatic forces. The chemical extraction solvents are hexane, benzene and ether. The first one is the most popular and inexpensive but is a good solvent only for lipids of low polarity. Benzene is not in used anymore since it is now considered as a potent carcinogenic substance. Benzene is now replaced by toluene.

2.6.2.1 Hexane Extraction Method

Hexane solvent can be used together with a mechanical extraction method. First pressing the oil using an expeller, then the remaining product can be mixed with hexane to extract all the oil content. After that, oil and hexane are separated by distillation. Other solvents can also be used e.g. ethanol and hexane-ethanol mixture. With these solvents it is possible to obtain up to 98% quantitative extraction of purified fatty acids.

2.6.2.2 Soxhlet Extraction

Oils from algae are extracted through repeated washing, with an organic solvent such as hexane or petroleum ether, under reflux in special glassware or Soxhlet extractor.

2.6.2.3 Folch Method

The tissue is homogenized with chloroform/methanol (2:1 v/v) for 1 ½ h. The liquid phase is recovered by filtration or centrifugation, and the solvent is washed with 0.9% NaCl solution. The lower chloroform phase containing lipids is evaporated under vacuum in a rotary evaporator.

2.6.2.4 Supercritical Fluid Extraction

In supercritical CO₂ extraction, CO₂ is liquefied under pressure and heated to the point that it has the properties of both liquid and gas. This liquefied fluid then acts as a solvent for extracting the oil. CO₂ is the most commonly used supercritical solvent because the compounds can be obtained without contamination by toxic organic solvents and without thermal degradation.

2.7 Yield Parameters

2.7.1 Lipid Content

Many microalgae species can be induced to accumulate high amounts of lipids thus contributing to a high oil yield. Typically, lipid content of microalgae oil is recorded as percentage of cell dry weight (% cdw).

2.7.2 Lipid Productivity

Lipid productivity can be calculated as the product of biomass productivity (g/L/day) and lipid content (% cdw), to give an indicator of oil produced on a basis of